Role of spermidine and nano calcium in the production of essential oil of *Mentha piperita* L. under salt stress

Synopsis

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Introduction

Mentha piperita L. commonly known as peppermint, belongs to the family Lamiaceae, is a highly valued popular medicinally important plant for its menthol rich essential oil (EO). Essential oil is a mixture of heterogeneous organic, aromatic and volatile liquid obtained from bark, flower, bud, rhizomes, root, leaves, stem, wood, and even from whole plant. These are the complex secondary metabolites which are derivatives of terpene, terpanoid and phenyl perpanoids (Brenes & Roura, 2010; Angane et al. 2022). Essential oil of peppermint is known for its coolness, freshness and fragrance due to the presence of active constituents viz menthol, and menthone, it is widely uses in cosmeceuticals, personal hygiene products, foods and pharmaceutical products besides being used as an astringent, antiseptic, antiemetic, vermifuge, diaphoretic and analgesic (Tiwari, 2016) as well as peppermint tea is used to treat coughs, bronchitis and inflammation of oral mucosa and throats. (Chawala & Thakur, 2013)

Although, India is the largest producer and dominant source of peppermint oil in the world. According to Singha et al. (2022), India produce 27,600 ton peppermint oil per year. The benefit-cost ratio of mentha cultivation (4.78) has found to be higher than wheat (2.47), potato (2.26), paddy (2.24), and maize (2.05) cultivation (Gill & Singh, 2014). Hence, the Mentha has turned out to be a favorite and profitable cash crop among farmers. So, the increased crop productivity of peppermint plant is highly desirable through different agricultural techniques.

Although it is highly productive crop yet does not fulfill the need of consumption that's why synthetic peppermint frequently reported in market (Taylan et al. 2021). On the other hand its productivity is also diminish under environmental stress like drought, salinity, heat and others. In which salinity is one of them that significantly hinder quantity and quality of peppermint oil (Qureshi et al. 2005). It has been estimated that more than 50% of the arable land would be salinized by the year 2050 (Chele et al. 2021). India stands second after China among the Asian countries possessing high saline and alkaline lands (Kumar et al. 2021). In India, the area under salt-affected soils is about 6.73 million ha with states of Gujarat (2.23 m ha), Uttar Pradesh (1.37 m ha), Maharashtra (0.61 m ha), West Bengal (0.44 m ha) and Rajasthan (0.38 m ha) together accounting for almost 75% of saline and sodic soils in the country (Sharma & Singh, 2015; Mandal et al. 2018).

According to Neeraj et al. (2019) several district of Uttar Pradesh viz Mainpuri, Kannuaj, Etawah, Auriya, Raebareli, Pratapgarh, Sultanpur, Jaunpur, Sant Ravidas nagar, Azamgarh, Ghazipur, Mau and Candauli are affected with salinity stress.

Plants grow in high salinity stress face problems viz membrane disintegration, loss of metabolic function, ion leakage, increase reactive oxygen species, DNA defragmentation, and subsequent cell death as well as yield attribute (Hasegawa et al. 2000).

To counteract the harmful effects of environmental stresses, plants boost the synthesis of various growth and stress-related regulators and signalling molecules. Under abiotic stress, several signalling molecules such as calcium, nitric oxide, cyclic adenosine monophosphate, and others and stress regulators, including absicsic acid, jasmonic acid, brassinosteroid, ethylene, and polyamines have been identified. In which polyamines (putrescine, spermidine, and spermine) are considered to be a new class of growth regulators in plants (Xu et al. 2014). They are ubiquitous natural compound with low molecular weight and open-chain nitrogen structure present in bacteria, animals, and plants (Alcázar et al. 2010). They show tissue and organ-specific distribution patterns in plants. In plants, two precursors viz., l-ornithine and l-arginine, can transform into putrescine which further converts into spermidine and spermine. Polyamines which is positively charged bind to macromolecules such as DNA, RNA and proteins, stabilize and involved in diverse processes, including regulation of gene expression, translation, cell proliferation, modulation of cell signalling, and membrane stabilization (Tyagi et al. 2022).

Spermidine ($C_7H_{19}N_3$) is one of the polyamine that is synthesized from putrescine by spermidine synthase (SPDS). The application of different types and concentrations of exogenous spermidine has been reported to alleviate the effects of salinity stress on various plants, and reduce damages (Verma and Mishra, 2005) by increasing proline content, antioxidant enzyme activity, decrease ROS level, reduce chlorophyll destruction and increased photosynthetic rate (Li et al. 2015).

Plant nutrient are also necessary for plant growth, reproduction, metabolism, biotic and abiotic stresses. There are twenty elements to be essential for plant that are classified as macro nutrient viz nitrogen, phosphorus, potassium, calcium, sulphur, magnesium, carbon, oxygen and hydrogen micronutrient viz iron, boron, chlorine, manganese, zinc, copper, molybdenum and nickel. In which,

calcium play a dual role as secondary macronutrient and secondary messenger under drought and salinity stress.

In recent year soil is not much fertile as earlier. Deficiency of several nutrient are reported frequently. Reason of deficiency of nutrient are many viz nutrient leaching, absorption surface area, solubility and many more. Recently, nano-fertilizers are using as pioneer in solving the problems in agriculture in order to increase crop production, quality, and boost nutrient uptake efficiency (Froggett, 2009). Nano-fertilizers perform extremely efficiently in contrast to traditional fertilizers due to their high surface area to volume ratio and transport to different parts of the plant. It has been reported that nanoparticles can reduce the damages caused by environmental stresses, as they affect hormonal concentrations, antioxidant enzyme activity, ion homeostasis, gene expression, and defense system functions (Noohpisheh et al. 2021; Zulfiqar & Ashraf, 2021; Nasrallah et al. 2022).

Calcium ion is well known for its essential role as a mediator of signals and in regulating as well as specifying the cellular responses to abiotic stresses. Calcium nanoparticles is a promising a new nano growth promoter and nano-fertilizers for agricultural use. In recent year, nano calcium found to be significantly reduced the negative effect of salt stress by provide structural support on the plant cell walls, regulate SOS (salt overly sensitivity) pathway, reduce reactive oxygen species level, reduce hydrogen peroxide, and crosstalk with stress-related genes under salt stress (Liang et al. 2018).

Thus, improved varieties and novel strategies which are tolerant to salt stress are the ultimate solution for the large-scale commercial cultivation of important medicinal and aromatic plants like *Mentha piperita* L. There are limited available reports on the role of spermidine and nanocalcium to mitigating negative effect of salt stress on the said plant. Therefore, we hypothesized that the foliar application of spermidine and nano calcium can mitigate the negative effect of salt stress and open new ways to deal with environmental stress like salinity.

Need of this research

- In the future, salinity is expected to increase, which will inevitably increase the severity of abiotic stresses. Therefore, salinity-tolerant crop varieties are urgently needed to be ensured.
- The production of peppermint oil and its active constitutes viz menthol is lower than the demand of its essential oil and menthol content. EO and its active constituents are used in various

industries i.e. toothpaste, pharmaceuticals, cosmetic products etc. Therefore, synthetic peppermint oil is using instead of natural peppermint oil.

- The demand of natural peppermint oil is high in global market.
- High yield of peppermint will de benefited to farmers economically.
- Nano calcium can most effective to enhance the production of essential oil and its active constituents because calcium work as limiting nutrient for the growth and development of plant after nitrogen.
- Future findings of this study will facilitate us to understand the mechanism of spermidine and nanocalcium under salt stress.

Objectives

Keeping this in mind, it is decided to investigate the role of spermidine and nano calcium in the production of essential oil of *Mentha piperita* L. under salinity stress. To achieve the goal, the following experiment will be conducted on *Mentha piperita* L. at Botanical Garden, DEI, Agra Uttar Pradesh India

- To study the effect of foliar application of spermidine on the growth, physiological, biochemical and yield parameters in *Mentha piperita* L.
- To find out the effect of foliar application of spermidine on growth, physiological, biochemical and yield parameters in *Mentha piperita* L. under salt stress.
- To work out the optimum concentration of leaf applied nano calcium on the growth, physiological, biochemical and yield parameters in *Mentha piperita* L.
- To investigate the effect of foliar application nano calcium on the growth, physiological, biochemical and yield parameters in *Mentha piperita* L. under salt stress.
- To find out the best concentration of combined foliar application of spermidine and nano calcium on the growth, physiological, biochemical and yield parameters in *Mentha piperita* L. under salt stress

Review of literature

Systematic position of the plant

According to system of classification of Arthur Cronquist (1981). *Mentha piperita* occupies following

systematic position:

Kingdom	Plantae
Sub kingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae
Genus	*Mentha*
Species	*piperita* L.

Fig:1 *M. piperita* at Botanical garden, Agra

Description of the plant

Mentha piperita L. is an aromatic herb that is a sterile hybrid between *Mentha spicata* and *Mentha aquatica* (Croteau et al. 2005; Gill & Singh, 2014). It is distributed mostly in the Northern hemisphere. In India, it is largely confined to North India *viz* Uttar Pradesh, Punjab, and Haryana. *Mentha piperita* is the second most highly cultivated after *Mentha arvensis* in India. It is a herbaceous, rhizomatous, perennial plant that grows to be 30 – 90 cm tall with smooth and square stems. The runners are wide-spreading fleshy and bear fibrous roots. The leaves can be 4 –9 cm long and 1.5– 4 cm broad. They are dark green with reddish veins, with an acute apex and coarsely toothed margins. The flowers are purple, 6–8 mm long, with a four-lobed corolla they are produced in whorls with verticillasters inflorescence. Flowering season lasts from mid-to-late summer.

History of origin

Mentha is indigenous to Europe and the Middle East. It is mainly cultivated in Argentina, Angola, Australia, Brazil, Bulgaria, China, Czechoslovakia, France, Hungary, India, Italy, Paraguay, Switzerland, Thailand, and the United State of America. Now it is widely spread and cultivated in many regions of the world. In India, the majority of this crop is grown in Uttar Pradesh (Chandausi and Barabanki), Punjab, Haryana, and Rajasthan (Verma & Singh, 2022). India is the largest producer

(produces 80% of the world supply) and exporter (exports 75% of output) of mentha oil in the world since 1993 (Agarwal et al. 2022).

Varieties of *Mentha piperita* L.

The cultivars of *Mentha piperita* include many cultivars viz CIM-Indus (0.25%), CIMAP-Patra (0.35%), CIM-Madhurus (0.45%), and Kukrail (oil content- 0.50%) (Developed by CSIR-CIMAP).

The breeding lines of *M. piperita* are MPS-25 (0.30%), MPS-9 (0.35%), MPS-36 (0.35%) MPS-5 (oil content-0.40 %), MPS-6 (0.45%), MPS-16 (0.45%), MPS-20 (0.45%) are originated by CSIR-CIMAP, Lucknow (Kumar et al. 2015).

A**ctive constituents of essential oil of *Mentha piperita* L.**

Essential oil is extracted from its aerial parts such as stems and leaves. Its essential oil has 1,8 cineole (0.17-62.16%), Menthol (9.88-53.28%), Linalool (0.14-51%), Menthone (0.8- 49.89%), Methyl acetate (0.96-35.01%), Limonene (0.10-37.18%), Carvone (0.20-34.94%), α -Terpinene (0.10-19.7%), Pulegone (0.42-14.8%), Menthafuran (0.31-11.95 %), Iso-menthone (2.35-11.80%), D-Limonene (1.30-11.20%), Neo-isomenthol (0.2-6.55%), α -Pinene (0.12-4.8%), p-cymene (0.06-2.78%), Piperitone (0.1-2.1%), Myrcene (0.2-1.21%), Camphene (0.203-1.2%) and Isopulegol (0.41-0.53%) (Mahendran & Rahman, 2020).

Industrial uses

It has a wide range of applications in the pharmacy, food, and cosmetic industry. It is also used in oral hygiene products and bad breath remedies like alcoholic beverage, baked goods, candy, chewing gums, desserts, food flavoring, mouthwash, mouth and tongue spray, toothpaste, and more generally in tobacco and supari.

Pharmacological properties

Menthol has various biological properties, such as sedative, anesthetic, antiseptic, vasodilator, analgesic, local anesthetic, antimicrobial, and antipruritic (Trevisan et al. 2017; Haydari et al. 2019). Menthol is also used in colic pain disorders and cough and cold relieving remedies.

Salinity stress

Bistgani et al. (2019) found that salinity stress decreased significantly the yield of both species *Thymus vulgaris* and *Thymus daenensis*, whereas improved the phenolic contents and antioxidant

capacity of thyme plants.

Ghorbani et al. (2018) studied that increasing the salinity stress significantly impressed all of the studied morpho-physiological traits in peppermint and reduced them. Although most of the essential oil components were decreased under salinity stress but some components of essential oil such as menthon were increased under salinity stress conditions.

Khorasaninejad et al. (2010) investigated that an increase in the salinity (0, 50, 100, and 150 mmol L^{-1} NaCl) resulted in a reduction in height of stem/ root, fresh weight, and dry weight of stem/ root, internodes length, total biomass, and essential oil percent and yield. They also mention that peppermint did not tolerate 150 mmol L^{-1} NaCl and died under this condition.

Loko et al. (2022) researched that salt stress reduces the growth of African basil plants due mainly to Na+ ion toxicity. The ionic selectivity ratio (K^+ /Na^+) rather than the K^+ ion content plays an important role in the response of basil plants to salt stress, while both proline and soluble sugars accumulation may contribute to osmotic adjustment.

Pour-Aboughadareh et al. (2021) reported that salinity treatment significantly decreased the root and shoot dry weights, photosynthesis rate, stomatal conductance, transpiration rate, shoot K^+ content, root K^+/ Na^+, shoot K^+/ Na^+, root-to-shoot Na^+ translocation, and root-to-shoot K^+ translocation, but resulted in increased root Na^+ content, root K^+ content, and shoot Na^+ content in different wheat genotype.

Spermidine (Spd)

Al-Mushhin, (2022) studied and suggest that K (100 mg KCl / kg soil) and spermidine (100 μM spermidine) application protect the growth and photosynthesis from salinity (100 mM NaCl) induced oxidative damage by up-regulating the ion homeostasis, antioxidant system, osmolyte accumulation, and secondary metabolite synthesis.

Karaman et al. (2008) concluded that exogenous polyamines (0.25, 0.5, 1, and 10 mM) can promote oil yield in aromatic plants spermidine, spermine and putrescine may affect essential oil accumulation and composition differently in *Ocimum basilicum* L.

Mustafavi et al. (2016) studied that the foliar application of spermidine and spermine (spm) (1mM) and inferred that polyamines which is economical and environment friendly alternative can be

implicated to improve the productivity of valerian under drought stress.

Orabi et al. (2020) studied that exogenously applied three concentrations of spermidine (25, 50, and 100 mg/L) with cold stress. Growth parameters and SPAD values of the two tomato cultivars relative to corresponding control plants were increased. Moreover, all treatments caused marked decreases in MDA and methylglyoxal (MG) values accompanied by significant increases in ascorbate peroxidase (APX), Phenylalanine ammonia-lyase (PAL) catalyzes and Glyoxalase I activities relative to control plants. All treatments almost caused significant increases in fruit yield (g/plant). It is worth mentioning that the highest recorded values were obtained in plants treated with 50 mg/L spermidine.

Qian et al. (2021) postulated that exogenously applied spermidine effectively alleviated the damage caused by salt stress (0.3% and 0.6%) by upregulating the relative electric conductivity, proline content, gas exchange, antioxidant enzyme activity, and expression of Catalase, peroxidase , MnSODM, P5CS, and Betaine-aldehyde dehydrogenase genes in *Gladiolus gandavensis* seedlings. However, when the seedlings were grown under 0.9% NaCl, no significant differences were found in the physiological and molecular responses between spermidine treated and non-spermidine treated plants. Therefore, they suggested that exogenous spermidine can efficiently counteract the adverse effect of low (0.3%) and moderate (0.6%) salt stress on *G. gandavensis* seedlings.

Nano calcium

Bachani et al. (2022) explained that Ca^{2+} dynamically affected diverse Na^{+} receptors, both directly and indirectly, through different phytohormones, SOS (Salt overly sensitive) pathways, and ROS (Reactive oxygen species) molecules. A significant number of salt-tolerant genes activated by Ca^{2+} signalling waves have been identified, which play a crucial role in preventing and reversing the damaging effects of excessive salinity. Hence, calcium can be considered one of the most versatile and imperative molecules, to mitigate the negative effect of salinity in plants.

Carmona et al. (2022) reviewed that nano calcium phosphate has greater solubility in water, large (specific) surfaces, and nanometric size have been functionalized and encouraging results on the superior nutrient use efficiency in several crops than their conventional counterparts.

Nasrallah et al. (2022) results showed that salinity had deleterious effects on plant yield with 55.9% the reduction compared to the control. On the other hand, calcium phosphate nanoparticle

dramatically improved plant yield by 30% compared to conventional fertilizers under salinity stress. This improvement could be attributed to significantly higher enhancement in total soluble sugars, antioxidant enzymes, proline content, and total phenolics recorded use of nano-calcium compared to conventional use under salt stress.

Sajyan et al. (2019) researched that salinity decreased fresh weight, and leaf area and increase electrolyte leakage in tomato while Lithovit® (LITHO) (nano-$CaCO_3$) helped to increase leaf area, dry mass, total chlorophyll content, and total soluble solid and decreased electrolyte leakage.

Upadhyaya et al. (2017) researched that calcium phosphate nanoparticles have shown to promote growth of shoot and root in rice plants. It modulates ROS and antioxidant responses by stimulating antioxidant system including enzymes and metabolites.

Proposed Methodology

Experiment location

The experiments will be conducted at the Botanical garden, Dayalbagh Educational Institute, Dayalbagh Agra, Uttar Pradesh, India. Physio-biochemical analysis of soil will be done for the experiment. After completion of all treatments, the following parameters will be analyzed.

1. **The growth parameters will be**

- Plant height
- Shoot fresh weight
- Shoot dry weight
- Root fresh weight
- Root dry weight
- Leaf area

2. **Physiological and biochemical parameters will be**

- Total chlorophyll content by (Maclachlan and Zalik, 1963)/ SPAD (Soil Plant Analysis Development)
- Total carotenoid content (Maclachlan and Zalik, 1963)
- Photosynthetic rate by IRGA (Infrared Gas Analyzer)
- Stomatal conductance by IRGA (Infrared Gas Analyzer)

- Ion percentage (Na^+ and K^+ percentage)
- Proline content (Bates et al. 1973)
- Relative water content (RWC) (Kaya et al. 2013)
- Hydrogen peroxide content (Aftab et al. 2011)
- Nitrate reductase activity (Jaworski, 1971)
- Carbonic anhydrase activity (Dwivedi and Randhwa,1974)
- Malondialdehyde (MDA) contents (Perveen et al. 2016)
- Cell membrane permeability (Aftab et al. 2011)
- Antioxidant assay (Chrysargyris et al. 2017)
- SOD(superoxide dismutase EC1.15.1.1)
- POD (peroxidase EC1.11.1.7)
- CAT (catalase EC1.11.1.6)

3. The yield Parameters

Essential oil per extracted by hydro-distillation using Clevenger's apparatus and then quantified gravimetrically according to Guenther (1972).

Oil content will be estimated by the following formula

$$\text{Oil } (\%\frac{v}{w}) = \frac{\text{Observed volume of oil}}{\text{Weight of sample}} \times 100$$

- Essential oil content per plant
- Essential oil yield per plant
- Herbage yield per plant

Active constituents will be analyze by the using of Gas Chromatography-Mass Spectroscopy (GC-MS).

- Menthol content per plant
- Menthol yield per plant
- Menthone content per plant
- Menthone yield per plant
- Pulegone content per plant

- Pulegone yield per plant
- Menthofuron content per plant
- Menthofuron yield per plant

4. Statistical analysis

Statistical analysis will be done by SPSS statistical software.

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